

CLAIMS

1. A method for producing a gene tag for eukaryotic cells, which comprises the steps of:
(1) linking an RNA linker, comprising a type IIs endonuclease recognition sequence, to the CAP
5 site of an RNA;
(2) synthesizing a cDNA using the resulting RNA of (1) as a template; and
(3) reacting the resulting cDNA of (2) with a type IIs endonuclease that recognizes the recognition
sequence in the RNA linker, and thereby generating the gene tag.

10 2. The method of claim 1, wherein step (2) comprises synthesizing a cDNA by the steps
of:
(i) synthesizing a first strand of the cDNA using a primer that anneals to an arbitrary region of the
RNA; and
(ii) preparing a double-stranded cDNA by synthesizing a second strand of the cDNA using a
15 primer that anneals to a region of the first strand which has been synthesized using the RNA linker
as a template.

20 3. The method of claim 2, wherein the primer that anneals to a region of the first strand
which has been synthesized using the RNA linker as a template has a label that binds to a solid
phase or is immobilized onto a solid phase, wherein the method further comprises the step of
recovering the double-stranded cDNA by recovering the solid phase.

25 4. The method of claim 3, wherein the solid phase is recovered before or after reaction
with the type IIs endonuclease.

5. The method of claim 1, wherein the RNA linker further comprises a type II
endonuclease recognition sequence.

30 6. The method of claim 1, wherein the method further comprises the step of generating a
ditag by linking the end of a gene tag cleaved by the type IIs endonuclease to the end of another
gene tag cleaved by the type IIs endonuclease.

35 7. The method of claim 6, wherein the method further comprises the step of amplifying
the ditag using a primer that anneals to the RNA linker.

8. The method of claim 1, wherein the method further comprises the step of ligating an

adapter having an arbitrary nucleotide sequence to the end of a gene tag cleaved by the type IIs endonuclease and amplifying the gene tag using primers that anneal to the RNA linker and the adapter.

5 9. A method for producing a concatemer of gene tags, wherein the method comprises the step of linking multiple gene tags generated by the method of claim 1.

10 10. A method for producing a concatemer of gene tags, wherein the method comprises the step of linking multiple ditags generated by the method of claim 6.

11. A method for determining the nucleotide sequence of a gene tag, wherein the method comprises the step of determining the nucleotide sequence of a concatemer produced by the method of claim 9 or 10.

15 12. A reagent kit for producing a gene tag, wherein the kit comprises:
(a) an RNA linker that comprises an oligonucleotide comprising a type IIs endonuclease recognition sequence;
(b) a reagent for linking the RNA linker with the CAP site of an RNA;
(c) a primer for cDNA second strand synthesis, which comprises an oligonucleotide that anneals to
20 a cDNA synthesized using the RNA linker as a template; and
(d) a primer for cDNA first strand synthesis.

13. The kit of claim 12, wherein the primer for cDNA first strand synthesis is selected from the group consisting of:
25 (i) a random primer;
(ii) an oligo dT primer; and
(iii) a primer comprising a nucleotide sequence complementary to a particular mRNA.

14. A method for obtaining an expression profile of a gene in eukaryotic cells, wherein the
30 method comprises the steps of:
(1) producing a gene tag by the method of claim 1;
(2) determining the nucleotide sequence of the gene tag of (1); and
(3) obtaining the expression profile by relating the determined nucleotide sequence to its frequency of occurrence.

35 15. A database of gene expression profiles constructed by accumulating information of

gene expression profiles obtained by the method of claim 14.

16. A method for analyzing gene expression profiles, wherein the method comprises the step of obtaining gene expression profiles from different types of cells by the method of claim 14,
5 comparing the gene expression profiles and selecting a gene tag whose frequency of occurrence differs among the cells.

17. A method for determining the transcriptional start site of a gene, wherein the method comprises the steps of:

- 10 (1) producing a gene tag by the method of claim 1;
(2) determining the nucleotide sequence of the gene tag of (1); and
(3) mapping the determined nucleotide sequence onto a genomic nucleotide sequence and identifying a region where the nucleotide sequences match as the transcriptional start site of the gene.

15 18. The method of claim 17, wherein the primer for cDNA first strand synthesis comprises a nucleotide sequence selected from the nucleotide sequence of a particular gene, wherein the method comprises determining the transcriptional start site of the gene.

20 19. A primer set for cDNA synthesis, wherein the primer set comprises a 3' primer that anneals to an arbitrary portion of a cDNA and a 5' primer for synthesizing a cDNA comprising a nucleotide sequence, or the complementary sequence thereto, determined by the steps of:
(1) producing a gene tag by the method of claim 1; and
(2) determining the nucleotide sequence of the gene tag of (1).

25 20. The primer set of claim 19, wherein the 3' primer is selected from the group consisting of:

- (i) an oligo dT primer;
(ii) sequence information on a cDNA fragment; and
30 (iii) a primer comprising the nucleotide sequence of a gene tag adjacent to the type II endonuclease recognition sequence in the cDNA or the complementary sequence thereto.

21. A method for synthesizing a full-length cDNA, wherein the method comprises the steps of:

- 35 (a) carrying out complementary strand synthesis using an RNA or cDNA as a template and using a 3' primer comprising an oligo dT primer and a 5' primer for synthesizing a cDNA comprising a

nucleotide sequence, or the complementary sequence thereto, determined by the steps of:

- (1) producing a gene tag by the method of claim 1; and
 - (2) determining the nucleotide sequence of the gene tag of (1); and
- (b) recovering a synthesized DNA as the full-length cDNA.

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22. A full-length cDNA obtained by the method of claim 21.

23. A polypeptide comprising an amino acid sequence encoded by the full-length cDNA of claim 22.

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24. An antibody which recognizes the polypeptide of claim 23.

25. A vector carrying and capable of expressing the coding region of the full-length cDNA of claim 22.

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26. A transformant comprising and capable of expressing the vector of claim 25.

27. A method for producing the polypeptide of claim 23, wherein the method comprises the step of culturing the transformant of claim 26 and collecting an expressed product.

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28. A method for producing the polypeptide of claim 23, wherein the method comprises the steps of:

- (i) contacting an element supporting *in vitro* translation with a DNA construct comprising the coding region of the full-length cDNA of claim 22 operatively linked to a promoter; and
- (ii) collecting an expressed product.

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29. A method for synthesizing a cDNA comprising a nucleotide sequence from the 5' end of an mRNA, wherein the method comprises the steps of:

- (a) carrying out complementary strand synthesis using an RNA or cDNA as a template and using a 3' primer comprising a nucleotide sequence complementary to an arbitrary region of an mRNA of interest and a 5' primer for synthesizing a cDNA comprising a nucleotide sequence, or the complementary strand thereto, determined by the steps of:

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- (1) producing a gene tag by the method of claim 1; and
 - (2) determining the nucleotide sequence of the gene tag of (1); and
- (b) recovering a synthesized DNA as the cDNA comprising a nucleotide sequence from the 5' end of an mRNA.

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30. A method for determining the 5' nucleotide sequence of an mRNA, wherein the method comprises the step of determining the nucleotide sequence of the cDNA recovered by the method of claim 29.